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A Human Cell Line Constitutively Expressing HIV-1 *Gag* and *Gag-Pol* Gene Products

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A human cell line constitutively expressing the HIV-1 *gag* and *pol* genes products was established. The cell line was established by stably transfecting 293 cells with a plasmid construct that expresses the HIV Gag and Pol and can confer the transfectants resistant to mycophenolic acid. Particles generated from transient expression of the plasmid construct were noninfectious when pseudotyped with HIV envelope or with amphotropic murine leukemia virus envelope proteins. However, virus-like Gag particles produced by the stable cell line were appropriately processed, exhibited a wild-type retrovirus particle density, and possessed significant reverse-transcriptase (RT) activities. Continuous passage of the cell line either in the presence or absence of mycophenolic acid had no major effects on the Gag processing efficiency, particle assembly, or RT activity release. It was also demonstrated that the proteolytic processing of the virus-like particles released from the cell line was inhibited by an HIV protease inhibitor, saquinavir. The establishment of a stable cell line producing noninfectious but proteolytically processed HIV Gag particles offers a safe, convenient tool for biochemical and immunological analysis of virus-like particle assembly and is very useful for the development of anti-HIV protease drugs. ***J. Med. Virol. 57:17–24, 1999.***

KEY WORDS: human immunodeficiency virus; Gag; Gag-Pol; HIV protease inhibitors

INTRODUCTION

Assembly of human immunodeficiency virus particles occurs at the plasma membrane where the primary structural protein $Pr55^{gag}$ and the $Pr160^{gag\text{-}pol}$ assemble into virus particles and bud out from the cell surface with incorporation of envelope glycoproteins [Stephens et al., 1988; Cann et al., 1989; Smith et al., 1993]. During or after virus budding the $Pr55^{gag}$ is proteolytically processed by the *pol*-encoded protease (PR) into (from N-terminus to C-terminus) p17 (matrix-associated [MA] protein), p24 (capsid-associated [CA] protein), p7 (nucleocapsid-associated [NC] protein), and the p6 subunit [Leis et al., 1988; Mervis et al., 1988; Overton et al., 1989; Henderson et al., 1992]. The *pol* gene products, besides protease, are reverse transcriptase (RT), RnaseH, and integrase, which are enzymes required for virus replication [Ratner et al., 1985]. The Pol polyprotein is initially translated as a $Pr160^{gag\text{-}pol}$ fusion protein by -1 ribosomal frameshifting at a frequency of about 5–10% [Jacks et al., 1988; Wilson et al., 1988]. During particle assembly, dimerization of the Gag-Pol induces activation of the embedded PR to cleave the $Pr55^{gag}$ and $Pr160^{gag\text{-}pol}$ [Iodawer et al., 1989; Lapatto et al., 1989; Navia et al., 1989; Kaplan et al., 1994; Quillent et al., 1996; Wondrak and Louis, 1996; Rasnick, 1997]. The PR-mediated maturation process of virions is essential for virus infectivity [Kohl et al., 1988; Gottlinger et al., 1989; Peng et al., 1989].

The Gag appears to play a central role in the process of virus assembly. The Gag-Pol is thought to be incorporated into assembling virions by virtue of its N-terminal Gag domain [Srinivasakumar et al., 1995]. To study the mechanism of HIV particle assembly and biological analysis of the HIV *gag* and *pol* gene products, a variety of systems, including baculovirus [Gheysen et al., 1989; Royer et al., 1992] and vaccinia virus [Karacostas et al., 1989; Haffar et al., 1990; Hu et al., 1990; Shioda and Shibuta, 1990; Smith et al., 1990; Jenkins et al., 1991; Vzorov et al., 1991], have been used to

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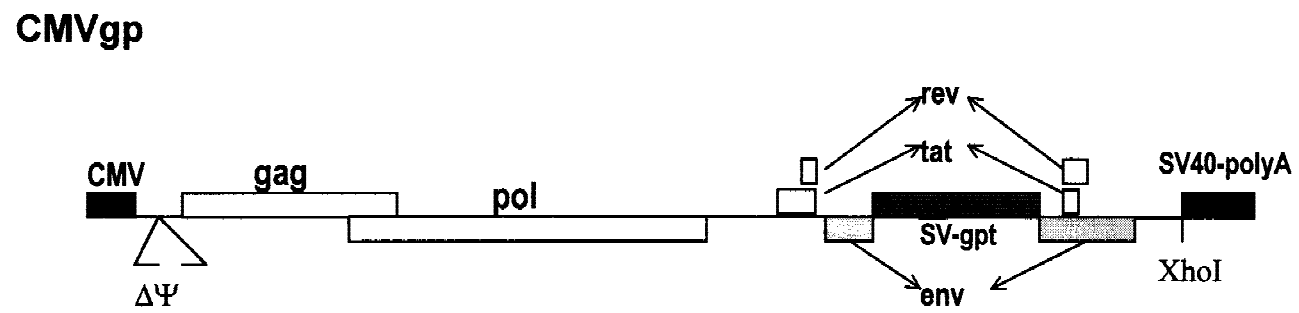

Fig. 1. The cDNA construct used to stably transfect the 293 cell line. The designated CMVgp was generated by modification of its parental construct HIVgpt as described in Materials and Methods. Expression of the HIV *gag* and *pol* is driven by a CMV promoter. The 3′ LTR and cDNA fragment downstream of the XhoI site were removed and replaced by an SV40 polyadenylation (polyA) signal. The deleted RNA packaging signal (psi) and the drug-resistant gene *gpt* are indicated.

express the $Pr55^{gag}$ and/or $Pr160^{gag\text{-}pol}$. Expression of HIV proteins in most of these systems, however, is transient. Development of a cell line stably expressing HIV Gag and Pol would be very helpful for a variety of HIV studies. Although mammalian cell lines stably expressing HIV $Pr55^{gag}$ and Env have been described [Haynes et al., 1991; Moosmayer et al., 1991; Krausslich et al., 1993], attempts to establish cell lines constitutively expressing wild-type HIV-1 *gag* and *pol* gene products were unsuccessful [Moosmayer et al., 1991; Krausslich et al., 1993], possibly due to cytototoxic effects of the expressed HIV gene products [Shoeman et al., 1990; Stewart et al., 1990; Kaplan and Swanstrom, 1991; Krausslich, 1991; Planelles et al., 1995]. A human cell line is described after stable transfection with an HIV-1 *gag* and *pol* gene expression construct could continuously produce proteolytically processed virus-like Gag particles containing significant RT activities.

MATERIALS AND METHODS

Plasmid Construction

As illustrated in Figure 1, the plasmid construct used to express the HIV-1 *gag* and *pol* gene products was originally derived from a replication-defective HIV proviral plasmid HIVgptPsi [Wang and Barklis, 1993], which is identical to the construct HIVgpt [Aldovini and Young, 1990], except that its major RNA packaging signal (psi) sequences between the splice donor (SD) site and the *gag* initiation codon site (nucleotides 748–786) were deleted. As previously described [Page et al., 1990], HIVgpt contains a deletion of the HIV-1 *env* coding region and an insertion of a fragment from the pSV2gpt that confers resistance to mycophenolic acid [Mulligan and Berg, 1981]. Flanking cellular and HIV-1 proviral sequences 5′ to SacI (nt 680), which included the 5′ long terminal repeat (LTR), were deleted from HIVgptPsi. Inserted at this site was a cytomegalovirus (CMV) promoter fragment excised from pcDNAINeo (Invitrogen, San Diego, CA). The 3′ LTR-containing fragment downstream of the XhoI site (nt 8896) was removed from the HIVgptPsi and replaced with a simian virus 40 (SV40) polyadenylation (polyA) signal. The resultant recombinant construct was designated CMVgp.

Cell Culture, Transfection, and Selection of Drug-Resistant Cell Colonies

HeLa cells and 293 human kidney cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum. Confluent 293 cells were split into 1:10 onto 10-cm dishes 24 hr before transfection. Twenty microgram plasmid DNA of CMVgp were transfected into 293 cells by the calcium phosphate precipitation method [Graham and van der Eb, 1973; Wang and Barklis, 1993]. At 48–72 hr posttransfection, culture media and cells were collected for protein analysis. For infectivity assays, 10 μg of plasmid DNAs of the HIVgpt or CMVgp were either transfected alone or cotransfected with 5 μg of plasmid DNAs of the murine leukemia virus (MLV) amphotropic envelope protein expression plasmid SV-A-MLV-env [Page et al., 1990] into 293 cells. Three days later, cell supernatants of 293 cells were used to infect HeLa cells. Adsorption of virus was allowed to proceed at 37°C in the presence of 4 μg/ml Polybrene. Three days after infection, cells were trypsinized and split 1:8 onto 10-cm dishes containing selection medium. The selective medium was made of penicillin plus streptomycin (GIBCO-BRL, Bethesda, MD), 50-μg/ml xanthine, 3-μg/ml hypoxanthine, 4-μg/ml thymidine, 10-μg/ml glycine, and 150-μg/ml glutamine [Wang and Barklis, 1993], plus 50-μg/ml mycophenolic acid (GIBCO-BRL). Cells were refed every 3 to 4 days with selection medium until colonies of drug-resistant cells formed. The number of colonies was converted to titer (infectious units/ml). HIVgpt could produce infectious viruses on coexpression with the SV-A-MLV-env, with a titer of 3530 ± 568 (infectious units/ml) in three independent experiments. This result agreed with the previous studies with COS7 cells expression system [Wang and Barklis, 1993]. Thus, expression of CMVgp in 293 cells could not produce infectious virus particles.

To establish a constitutive HIV Gag and Pol expression cell line, 20-μg linearized plasmid DNA of CMVgp were used to transfect 293 cells. Two days after transfection, cells were trypsinized and split 1:40 onto 10-cm

dishes containing selection medium as described above. Drug-resistant colonies were individually isolated, grown in 12-well plates, and screened for the released Gag protein levels by a dot-immunoblotting method using an anti-p24^{gag} monoclonal antibody [Talbot et al., 1984]. Clones giving the highest extracellular Gag protein signal were selected and expanded in a 10-cm dish containing selective medium plus 25-μg/ml mycophenolic acid.

Protein Analysis

Culture supernatants of either transient or stable CMVgp transfectants were collected, filtered through a 0.45-μm-pore-size filter, and centrifuged through a 2-ml cushion of 20% sucrose in TSE (10-mM Tris-HCl [pH 7.5], 100-mM NaCl, 1-mM EDTA, 0.1-mM phenylmethylsulfonyl fluoride [PMSF] at 4°C for 40 min at 274,000 × *g* [SW 41 rotor at 40,000 rpm]. Pellets were suspended in IPB (20-mM Tris-HCl [pH7.5], 150-mM NaCl, 1-mM EDTA, 0.1% sodium dodecyl sulfate [SDS], 0.5% sodium deoxycholate, 1% Triton X-100, 0.02% sodium azide) plus 0.1-mM PMSF. Cells were washed twice with 10 ml of ice-cold phosphate-buffered saline (PBS) and collected in 1 ml of 1 × PBS. Cells were then pelleted, resuspended in 1 ml of IPB plus 0.1-mM PMSF, and microcentrifuged at 4°C for 15 min at 13,700 × *g* (14,000 rpm) to remove cell debris. Cell lysates and supernatant samples were mixed with an equal volume of 2 × sample buffer (12.5-mM Tris-HCl [pH 6.8], 2% SDS, 20% glycerol, 0.25% bromophenol blue and 5% β-mercaptoethanol) followed by boiling for 4 to 5 min. Samples were subjected to SDS-PAGE and were electroblotted onto nitrocellulose membranes. Immunodetection of membrane-bound HIV Gag proteins followed the protocol as described previously [Chen et al., 1997]. The primary antibody was an anti-p24^{gag} monoclonal antibody at 1:5,000 dilution, and the secondary antibody was a sheep antimouse alkaline phosphatase-conjugated IgG antibody at 1:2,000 dilution. Color development substrates were nitroblue tetrazolium (NBT) and 5-bromo-1-chloro-3-indolyl phosphate (BCIP).

Sucrose Density Gradient Fractionation

Culture supernatants of stably transfected 293 cells were collected and filtered through a 0.45-μm-pore-size filter followed by ultracentrifugation through a 2-ml 20% sucrose cushion as described above. Pellets were suspended in TSE buffer, layered on top of a premade 20–60% sucrose density gradient, and subjected to centrifugation in an SW50.1 rotor overnight at 50,000 rpm (300,000 × *g*) at 4°C. Fractions were collected from top to bottom. Each fraction was measured for density and analyzed for Gag proteins by Western immunoblotting.

In Vitro RT Assays

Culture supernatants of transient or stable CMVgp transfectants were harvested, filtered, and pelleted as described above. Pellets were resuspended in 30 μl of TSE buffer. Ten μl of resuspended pellets were added to 40 μl of reaction cocktail containing 0.1% Triton X-100, 5-mM DTT, 10-mM $MgCl_2$, 50-mM Tris-HCl [pH 8.0], 1.2-mM poly(rA)-$(dT)_{15}$ (Boehringer Mannheim, Indianapolis, IN), and 25 μCi/ml [^{3}H]TTP (Amersham, Arlington Heights, IL). After incubation at 37°C for 2 hr, 5 μl of tRNA (10 mg/ml) was added to the reaction mixture, precipitated with ice-cold 10% trichloroacetic acid, and filtered with GF/C membranes. After washing and drying, filters were counted by a Beckman scintillation counter to determine the RT activity.

RESULTS

Establishment of a Human Cell Line Stably Expressing HIV Gag and Pol

To make an HIV Gag and Gag-Pol expression construct, we modified a previously constructed HIV replication-defective vector HIVgptPsi [Wang and Barklis, 1993]. As described above, the HIVgptPsi carrying an RNA packaging-defective mutation was derived from construct HIVgpt that contains a drug-resistant gene *gpt* in the *env* region. Previous studies have reported that transient expression of HIVgpt or HIVgptPsi could produce virus-like particles [Page et al., 1990; Wang and Barklis, 1993]. To prevent emergence of replication-competent HIV provirus through recombination event, the 5′ and 3′ LTRs of the HIVgptPsi were replaced by a cytomegalovirus (CMV) promoter and a simian virus (SV40) polyA signal, respectively. The resultant recombinant construct was referred to as CMVgp (Fig. 1).

Theoretically, particles released from CMVgp-expressing cells should be noninfectious since the CMVgp was derived from the noninfectious HIV expression construct HIVgpt. Moreover, the RNA encapsidation signal and both 5′ and 3′ LTRs essential for virus replication were deleted in CMVgp. Nevertheless, we tested whether expression of CMVgp in mammalian cells could produce infectious virus particles. To do so, plasmid DNAs of HIVgpt and CMVgp were either transfected alone or cotranfected with a murine amphotropic envelope expression plasmid SV-A-MLV-env [Page et al., 1990] into 293 cells. Virion-containing supernatants were assayed for RT activity and aliquots of equivalent RT activity for each transfection medium sample were used to infect HeLa cells. Infectivity assays were carried out as described above. As expected, no drug-resistant HeLa cell colonies were observed when culture supernatants derived from HIVgpt or CMVgp transfectants were used for infection assays. There were also no detectable infectious virus particles by cotransfection of the CMVgp and the envelope protein expression plasmid SV-A-MLV-env.

To establish a cell line that can stably express HIV Gag and Gag-Pol proteins, 293 cells were transfected with linearized CMVgp plasmid DNA and selection of drug-resistant colonies as described above. Initially, 25 drug-resistant cell clones were isolated and transferred onto 12-well plates containing selective media. Culture media from the individual clones were collected and the levels of released Gag proteins were screened by the

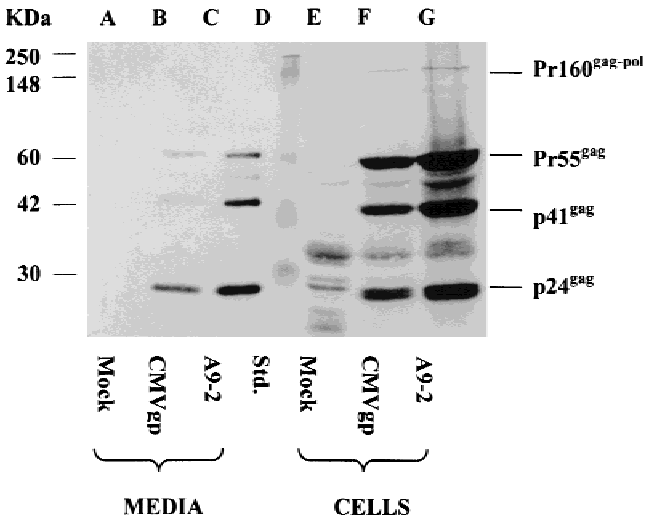

Fig. 2. Assembly and release of processed HIV particles. Culture supernatants and cells from transient CMVgp transfectants and the established cell line (A9-2) were collected and prepared for immunoblot analysis as described in Materials and Methods. Supernatant samples (lanes A ,B, and C, corresponding to 20% of the total sample) and cell samples (lanes E, F, and G, corresponding to 4% of the total sample) were fractionated by SDS-PAGE and were electroblotted onto a nitrocellulose membrane. HIV Gag proteins were immunodetected with an anti-p24^{gag} monoclonal antibody. Molecular size markers (See-Blue prestained standards [Novel Experimental Technology, San Diego, CA]; lane D) are indicated on the left, and HIV Pr160$^{gag\text{-}pol}$ and Gag proteins Pr55^{gag}, p41^{gag}, and p24^{gag} are shown on the right. Lanes A and E, mock; B and F, transient CMVgp transfectants; C and G, A9-2 cells.

dot-immunoblot method of Talbot et al. [1984]. Of seven positive clones, one clone, referred to as A9-2, which produced the highest level of HIV Gag, was selected and expanded in 10-cm dishes. Confluent A9-2 cells were split 1:10 every 3–4 days.

For analysis of the released HIV Gag particles, culture supernatants and cells of the A9-2 cell line were prepared for Western immunoblotting as described above. Results showed that the A9-2 cell line expressed and released proteolytically cleaved Gag proteins (Fig. 2, lanes C and G), in which most of the released Gag proteins were present as mature p24^{gag}, but some unprocessed (Pr55^{gag}) or incompletely processed (p41^{gag}) products were also observed, a processing pattern similar to that of transient expression samples (lanes B and F). These results indicated that the A9-2 cell line could stably express active HIV protease to cleave Gag precursors.

Previous studies have shown that medium supernatant virus proteins that were pelleted in a 40-min spin through a 20% sucrose cushion represent virus-associated proteins [Wang and Barklis, 1993]. To examine the particles produced by the expressed CMVgp in more details, a sucrose density gradient fractionation experiment coupled with Western immunoblotting analysis was performed. As shown in Figure 3, the peak p24^{gag} fraction 5 had a density of 1.16 g/ml, which is consistent with that for HIV particles [Bolognesi et al., 1973; Page et al., 1990; Wang and Barklis, 1993]. Additionally, the peak RT activity also banded at fraction 5 (data not shown). To further test that these particle released from the cell line were membrane enveloped, media supernatants from A9-2 cells were trypsin-treated as described previously [Wills et al., 1989; Weldon and Wills, 1993; Lee and Linial, 1994]. Results shown that Gag proteins were pelleted following trypsin treatment, suggesting that the Gag proteins were contained within membrane-enveloped particles (data not shown). Taken together, these results suggest that the Gag proteins released from the A9-2 cell line were particle-associated and that the levels of pelletable media Gag proteins reflected the levels of virus-like particle release from cells.

Stable Production of Particles Containing Significant RT Activities

The fact that A9-2 cells expressed the HIV Gag with a proteolytic processing pattern similar to that of the transiently transfected 293 cells (Fig. 2) suggests that the *pol*-encoded protease is constitutively expressed in A9-2 cells and functions properly. The incorporation of HIV *pol* gene products into virus-like particles was examined by in vitro reverse-transcription assay. Equivalent amounts of the identical transient and stable expression samples (Fig. 2, lanes B and C, respectively) were tested, and the counts per minute readings of incorporated nucleotide were 96,307 and 37,346, respectively, at least 25-fold over the background level (1,335 cpm for mock transfection). Data showing that the RT level produced by A9-2 cells was about threefold higher than that of CMVgp transfectants was compatible to the result of the Western immunoblot analysis in Figure 2, indicating that the observed particle-associated Gag protein signals in A9-2 sample (lane C) were relatively higher than that of the transient expression sample (lane B).

Previous attempts to stably transfect HIV *gag* and *pol* genes into mammalian cells led to the selection of cell lines producing only the unprocessed immature HIV virus-like particles [Haynes et al., 1991; Moosmayer et al., 1991; Krausslich et al., 1993]. Failure in construction of a stable cell line with enzymatically active HIV protease may be due to cytotoxic effects of the *pol* gene products [Shoeman et al., 1990; Stewart et al., 1990; Kaplan and Swanstrom, 1991; Krausslich, 1991]. To evaluate whether cell line A9-2 could stably express HIV *pol* gene products, both the RT activity level and the PR-mediated particle processing were monitored during continuous passage of the cell line. To do so, passage 3 of the A9-2 cell line was thawed and grown in 10-cm dishes containing media either with or without *gpt* selection. Culture supernatants derived from passages 5, 14, and 25 were collected and pelleted as described above. Viral pellets were resuspended in 50 μl of TSE buffer. Equivalent amounts (10 μl) of each sample were analyzed by RT assays and Western immunoblotting. As shown in Table I, samples from different passages (5, 14, and 25) all displayed an RT activity level at least 10-fold higher than that of the background. The level of particle production and the

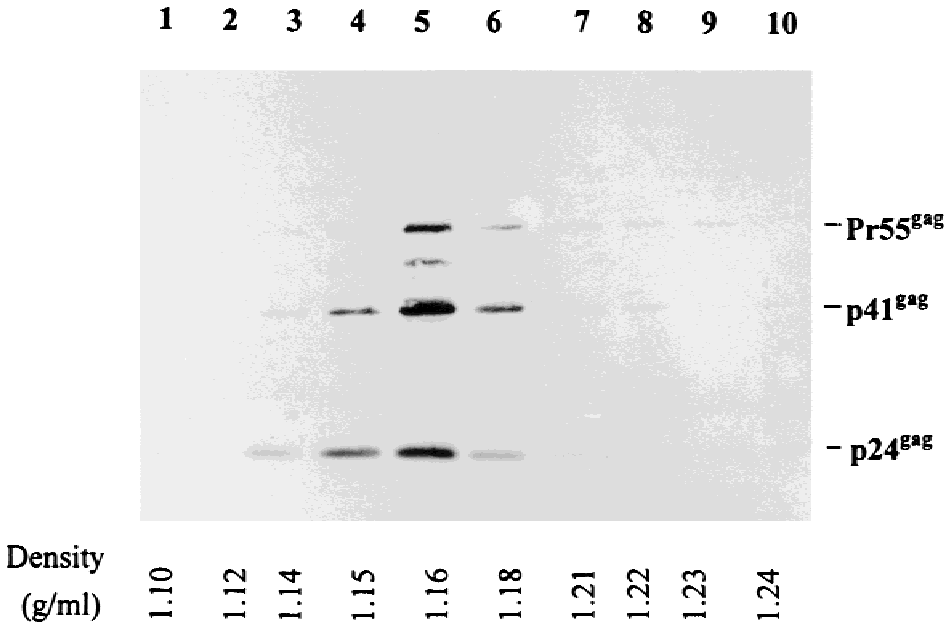

Fig. 3. Sucrose density gradient fractionation of HIV particles produced by A9-2 cells. Supernatants from A9-2 cells were collected and fractionated by a sucrose density gradient (20–60%) as described in text. Fractions were collected from top to bottom. Each fraction was measured for density and analyzed by Western immunoblotting. The peak Gag protein banded at fraction 5, which had a density of 1.16 g/ml. Densities for each fraction are indicated at the bottom.

TABLE I. Particle-Associated RT Activity Stably Produced by the A9-2 Cell Line[a]

Cells (passage number[b])	Incorporated cpm, *gpt* selection[c]	
	–	+
A9-2 (5)	62,567	38,478
A9-2 (14)	74,746	50,196
A9-2 (25)	55,754	48,528
Mock	3,156	
Recombinant HIV RT[d]	86,460	

[a]Culture supernatants of A9-2 cells were collected and prepared for RT assays as described Materials and Methods. Each sample used for RT assays corresponded to 20% of the total supernatant sample. Equivalent amounts of the same samples used for RT assays were analyzed by Western immunoblot as shown in Figure 4.
[b]During continuous subculture of the A9-2 cell line, culture supernantants from the 5th, 14th, and 25th passage were collected for RT assays.
[c]Presence (+) or absence (–) of *gpt* selection.
[d]Recombinant HIV-1 reverse transcriptase (Boehringer Mannheim) was used as positive control in parallel experiments. Counts per minute indicate the average RT activity per nanogram of the recombinant RT.

Gag processing efficiency also remained stable over a 4-month successive subculture of the cell line (Fig. 4). In the absence of *gpt* selection, however, A9-2 cells produced slightly higher RT activity and $p24^{gag}$ protein levels than those of their counterparts grown in the presence of *gpt* selection (Table I, Fig. 4). This may be due to a selective pressure effect on cell proliferation as cells grown in selective medium tended to be slightly less confluent than those grown in the absence of *gpt* selection. Nevertheless, the data shown above indicate that the A9-2 cell line was able to constitutively produce processed HIV virus-like particles that possessed significant RT activities.

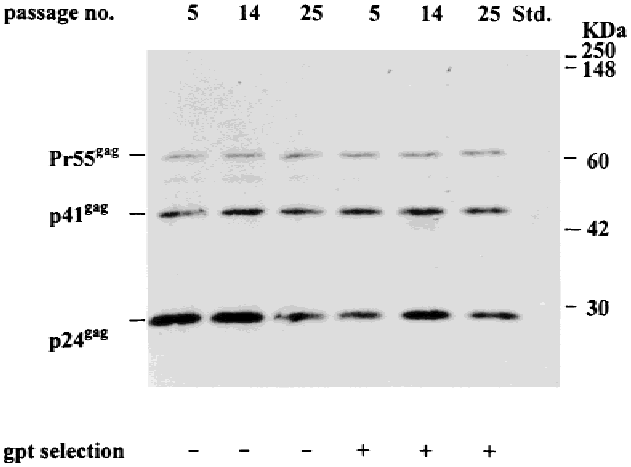

Fig. 4. A9-2 cells stably producing proteolytically processed HIV particles. Propagating A9-2 cells were split 1:10 every 3–4 days onto 10-cm dishes containing media either with (+) or without (–) *gpt* selection. Supernatants from the 5th, 14th, or 25th passage were collected and prepared for protein analysis as described in text. Each sample corresponding to 20% of the total sample was subjected to SDS-PAGE, and immunodetection of HIV Gag proteins was as described in Figure 2. Molecular size markers are indicated on the right and HIV Gag proteins $Pr55^{gag}$, $p41^{gag}$, and $p24^{gag}$ are shown on the left. Continuous passage of the cell line either in the presence (+) or absence (–) of *gpt* selection had no major effects either on the level of Gag particle production or on the efficiency of particle processing.

Inhibition of Particle Processing by Anti-HIV Protease Drug

Western immunoblotting analysis of the processing of the p24 capsid protein from the larger $Pr55^{gag}$ has been used to assess the inhibitory effects of saquinavir (RO 31-8958) on HIV protease. The inhibitory effects of

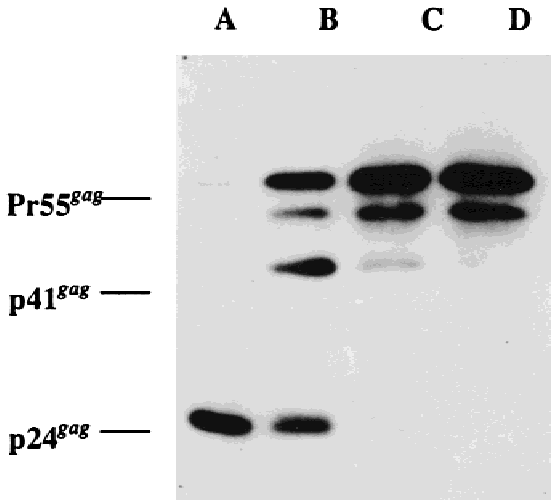

Fig. 5. Inhibition of HIV particle processing by saquinavir. Subconfluent A9-2 cells were split 1:10 onto four 10-cm dish plates containing media with different concentrations of saquinavir. Three days later, virus-containing media were harvested and prepared for Gag protein analysis as described in Figure 2. HIV Gag proteins Pr55, p41, and p24 are indicated on the left. Lanes A, mock-treated; B, 50 ng/ml; C, 100 ng/ml; D, 500 ng/ml of saquinavir.

saquinavir on the processing of HIV particles that were produced by the A9-2 cell line were examined. Culture supernatants were collected for protein analysis after a 3-day incubation with saquinavir. As shown in Figure 5, the proteolytic processing of released HIV Gag particles was inhibited by 100 ng/ml saquinavir as the $Pr55^{gag}$ was incompletely processed (lane B). When the concentration of saquinavir was increased to 500 ng/ml, no mature $p24^{gag}$ products were detected in the culture medium, with the $Pr55^{gag}$ representing the major species (lane D). This result is consistent with previous reports that in HIV-infected cells incubated with saquinavir, the $Pr55^{gag}$ was not processed to give to the $p24^{gag}$ [Roberts et al., 1990; Craig et al., 1991; Jacobsen et al., 1992].

DISCUSSION

We have established a human cell line (A9-2) constitutively expressing the HIV *gag* and *pol* gene products. HIV Gag particles produced by the A9-2 cell line were processed appropriately and possessed significant RT activities. So far, the cell line has been propagated over 6 months and there are no remarkable variations in the level of particle and RT activity production (Table I, Fig. 4). Previous attempts to establish a cell line stably expressing wild-type HIV *gag* and *pol* genes resulted in selection of clones only producing unprocessed HIV Gag particles [Haynes et al., 1991; Moosmayer et al., 1991; Krausslich et al., 1993], possibly due to a selective disadvantage for cells expressing active HIV protease. Although monkey cell lines stably transfected with HIV *gag* and *pol* genes were reported by one group [Carroll et al., 1994], most HIV particles produced by these cells were unprocessed or incompletely processed. These results support the suggestion that the HIV protease is cytotoxic to a variety of cells [Shoeman et al., 1990; Stewart et al., 1990; Kaplan et al., 1991; Krausslich, 1991] and have led to the development of an inducible HIV expression system to overcome this problem [Yu et al., 1996]. In addition to HIV protease, constitutive expression of Vpr has also been reported to be cytotoxic [Planelles et al., 1995]. It should be noted that HIV plasmid DNA sequences used in the study was derived from HXB2, which contains a truncated *vpr*. Our A9-2 cell line capable of stably producing HIV processed particles suggests that the 293 human kidney cells may be tolerant of constitutive expression of active HIV protease. Alternatively, the selected A9-2 cell line may be genetically mutated so that it can tolerate the constitutive HIV protease expression. Compared to its parental 293 cell line, the A9-2 cell line exhibited no morphologic changes under light microscope.

Our A9-2 cell line constitutively producing virus-like Gag particles will be very useful for immunological and biochemical analysis of virus-like particle assembly. One way to inhibit HIV replication is to block the process of virus maturation, which is mediated by the HIV protease. Besides AZT and other nucleoside analogue inhibitors [Mitsuya et al., 1985], HIV protease inhibitors appear to be another effective drugs in the inhibition of HIV replication [Moyle and Gazzard, 1996]. However, the new developed HIV protease inhibitors were mostly assayed by using chronically or acutely HIV-infected cells. Gag particles generated by A9-2 cells are processed in Gag and contain significant RT activities, but are noninfectious. Thus, the A9-2 cell line can become a convenient and safe tool in studying or screening the inhibitory effects of HIV protease inhibitory compounds.

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